

## Claims

1. A bovine beta-casein gene targeting vector comprising  
(1) a first region having a length of 5 to 12 kb which is  
5 homologous to the promoter and its flanking nucleic acid  
sequences of bovine beta-casein gene, and comprising exon 1,  
intron 1, and exon 2 of bovine beta-casein gene; (2) a  
region for cloning a nucleic acid coding for desired  
proteins; (3) a region for coding a positive selection  
10 marker; (4) a second region having a length of 2.8 to 3.5  
kb which is homologous to the nucleic acid sequences of  
bovine beta-casein gene, and comprising exon 5, 6, 7 and 8,  
and intron 5, 6 and 7 of bovine beta-casein gene; wherein  
the nucleic acid segment corresponding to the first region  
15 is located upstream to the nucleic acid segment  
corresponding to the second region in the 5'-3' arrangement  
of beta-casein gene.
- 20 2. The vector according to claim 1, wherein the length of  
the first region is 5.5 to 10kb.
3. The vector according to claim 1, wherein the length of  
the second region is 3.0 to 3.2 kb.
- 25 4. The vector according to claim 1, wherein the positive  
selection marker is selected from the group consisting of  
neomycin (Neo), hygromycin (Hyg), histidinol dehydrogenase  
gene (hisD) and guanine phosphosribosyltransferase (Gpt).

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5. The vector according to claim 1, wherein the vector further comprises a region for a negative selection marker.

5 6. The vector according to claim 5, wherein the negative selection marker is Diphtheria toxin (DT) gene.

7. A vector according to claim 1 or 5 which is pBCKI I, pBCKI II, pBCKIDT I or pBCKIDT II, is presented in FIG. 1, FIG.  
10 2, FIG. 16, or FIG. 3, respectively.

8. A bovine somatic cell which is beta-casein gene-targeted with the vector according to claim 1 or 5.

15 9. An embryo which is nuclear-transferred with the bovine somatic cell according to claim 8.

10. A method for producing a bovine beta-casein gene-targeted somatic cell which comprises the steps of (1)  
20 introducing the bovine beta-casein gene-targeting vector according to claim 1 or 5 into an bovine somatic cell;  
(2) occurring homologous recombination events in the bovine somatic cell; and (3) selecting the bovine beta-casein gene-targeted somatic cell with a desired gene by  
25 homologous recombination.

11. The method according to claim 10, wherein the vector in the step (1) is introduced into cells in form of linearized or deleted form lacking plasmid vector backbone.

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12. A method for generating transgenic cattle which  
comprise the steps of (1) introducing the bovine beta-  
casein gene-targeting vector according to claim 1 or 5 into  
a bovine somatic cell; (2) occurring homologous  
5 recombination events in the bovine somatic cell; (3)  
selecting the bovine beta-casein gene-targeted somatic cell  
with a desired gene by homologous recombination; (4)  
introducing the gene-targeted cell into a nuclear-removed  
bovine embryo to produce a nuclear-transferred embryo ; and  
10 (5) implanting the embryo into a recipient.

13. A method obtaining a large scale of desired proteins  
from milk of the transgenic cattle, in accordance with the  
method of claim 12.

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